

Impact of Diet on Lead in Blood and Urine in Female Adults and Relevance to Mobilization of Lead from Bone Stores

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We measured high precision lead isotope ratios and lead concentrations in blood, urine, and environmental samples to assess the significance of diet as a contributing factor to blood and urine lead levels in a cohort of 23 migrant women and 5 Australian-born women. We evaluated possible correlations between levels of dietary lead intake and changes observed in blood and urine lead levels and isotopic composition during pregnancy and postpartum. Mean blood lead concentrations for both groups were approximately 3 µg/dl. The concentration of lead in the diet was 5.8 ± 3 µg Pb/kg [geometric mean (GM) 5.2] and mean daily dietary intake was 8.5 µg/kg/day (GM 7.4), with a range of 2–39 µg/kg/day. Analysis of 6-day duplicate dietary samples for individual subjects commonly showed major spikes in lead concentration and isotopic composition that were not reflected by associated changes in either blood lead concentration or isotopic composition. Changes in blood lead levels and isotopic composition observed during and after pregnancy could not be solely explained by dietary lead. These data are consistent with earlier conclusions that, in cases where levels of environmental lead exposure and dietary lead intake are low, skeletal contribution is the dominant contributor to blood lead, especially during pregnancy and postpartum. **Key words:** adult females, bone, diet, lead isotopes, pregnancy. *Environ Health Perspect* 107:257–263 (1999). [Online 5 March 1999] <http://ehpnet1.niehs.nih.gov/docs/1999/107p257-263gulson/abstract.html>

Diet is currently considered the major contributing factor to blood lead levels due to decreasing lead concentrations in environmental and other traditional sources (1–4). We believe that although diet is the main exogenous source of lead for the subjects involved in this study, bone stores of lead are the predominant contributor to blood lead in cases where levels of lead in diets are low.

Blood lead prevalence surveys have demonstrated a considerable decline in blood lead levels over recent decades. For example, the National Health and Nutrition Examination Survey III showed a decrease in mean blood lead for the U.S. population from 13 µg/dl in 1978–1981 to 3 µg/dl in 1988–1991 (5). Similar trends have been observed in the United Kingdom (6), Sweden (7), Italy (8), and Spain (9). Explanations for declining blood lead have been the reduction or elimination of leaded gasoline for automobiles and lead solder in canned foods (1–4). The monitoring of dietary intake is especially relevant in studies involving subjects whose blood lead is <5 µg/dl, as is the case for most of the subjects in the present study.

Several recent studies have demonstrated that lead can be mobilized from the maternal skeleton during pregnancy and lactation in humans (10–12), in monkeys (13–14), and in rodents (15–16). In these studies it is essential to be able to distinguish between

lead from historical stores (such as bone) and lead from the current environment, especially in diets. In the animal studies, this problem can be fairly easily rectified with specific diets, but this is not the case for humans. In the human study we avoided this problem by measuring prospective lead isotope and lead concentration changes in the blood and urine and in environmental samples of migrated females whose bone stores of lead acquired over their lifetime are from isotopically different sources than their current Australian environment.

In this study we addressed the following questions: What is the impact of diet on blood and urine in adult females during pregnancy and postpartum and what is the relevance of these results to increased mobilization of lead from the skeleton during these times of physiologic stress?

Methods

Subjects. Our results are based on three groups of female adults, currently living in Australia, whose bone stores of lead acquired between 0 and 35 years of age are from isotopically different sources. The subjects included 23 migrants (15 pregnant subjects and 8 nonpregnant controls) and 5 pregnant Australian controls. None of the subjects were exposed to other potential lead sources—such as deteriorating leaded paints or older lead-bearing dusts released

by renovations and other activities—throughout the study period. Signed consent forms (translated into the subjects' native language) were obtained from each volunteer. This consent form was reviewed and approved by the Ethics Committee of St. Vincent's Hospital of Sydney, the University of Adelaide in Australia, and the U.S. National Institutes of Health. As part of the entry requirements into Australia, all subjects were declared medically fit.

Samples and collection. Venous blood and urine samples were collected according to strict protocols as soon as possible after arrival in Australia (no longer than 3 months) and then monthly thereafter for 4 months. Sampling was then quarterly until conception and remained quarterly for migrant subjects who did not conceive. For the pregnant migrants, blood and urine samples were collected monthly during gestation and during the postpregnancy period for up to 6 months. For the Australian control subjects, blood and urine samples were collected quarterly until parturition and then the same sampling regime was followed as for the migrants. A 6-day duplicate diet and environmental samples including drinking water, house dust, urban air, and gasoline were collected quarterly. Collection of the 6-day duplicate diet coincided with the

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The following reflects the individual professional position of the investigators and should not be interpreted as the official position of the U.S. Environmental Protection Agency.

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quarterly biological and environmental sampling. Each daily sampling was blended in a kitchen blender—several portions taken from each day's blended diet and composited and the 6-day composite was then blended in a laboratory blender.

Analytical methods. The dietary samples were analyzed by inductively coupled plasma mass spectrometry for calcium, magnesium, iron, barium, strontium, phosphorus, zinc, and copper. The methods for obtaining the lead isotope ratios and lead concentrations, including sample digestion, lead separation, thermal ionization mass spectrometry, and precision estimates, are detailed elsewhere (10–12,17–19), but are briefly described here. A justification for the small numbers of subjects for these studies is described by Gulson et al. (19). All sample preparation was performed in purpose-built low-contamination laboratories (clean rooms), incorporating features such as filtered air intake and laminar flow hoods.

Blood. To minimize sample heterogeneity, the total blood sample was predigested in ultrapure concentrated nitric acid and a <1-g aliquot was removed to a clean Teflon vessel. A ^{202}Pb spike solution of known isotopic composition and lead concentration was added to the aliquot to obtain the concentration of lead and isotopic composition of the unknown sample in one analysis (known as the isotope dilution method). Lead was separated from interfering elements, such as iron and zinc, by anion-exchange chromatography in a hydrobromic acid medium.

Urine. An aliquot of 5–10 ml was spiked with ^{202}Pb and digested in ultrapure nitric acid and the method of lead separation was the same as that for blood samples.

Dietary samples. After the sample was spiked with ^{202}Pb and predigested in ultrapure nitric acid, the sample was digested in a laboratory microwave oven. Lead was then separated as for blood samples.

Isotope ratio measurement. Fractions of the purified lead samples were loaded onto a rhenium filament using the silica gel technique and analyzed for lead isotope composition on a thermal ionization mass spectrometer (VG-ISOMASS 54E; VG Isotopes, Winsford, UK) run in fully automatic mode. Isotopic ratios were measured as $^{208}\text{Pb}/^{206}\text{Pb}$, $^{207}\text{Pb}/^{206}\text{Pb}$, and $^{206}\text{Pb}/^{204}\text{Pb}$. Precision estimates on the isotopic ratios have been defined by a repetition of the digestion/lead separation/mass spectrometry stages of the same samples of blood, urine, and water. The precision we allocate our data is $\pm 0.2\%$ (2σ) on the $^{206}\text{Pb}/^{204}\text{Pb}$ ratio, and $\pm 0.1\%$ on the $^{208}\text{Pb}/^{206}\text{Pb}$ and $^{207}\text{Pb}/^{206}\text{Pb}$ ratios. The $^{206}\text{Pb}/^{204}\text{Pb}$ ratio is normally discussed because it is easier to

understand differences at one decimal place than it is to understand up to four decimal places in, for example, the $^{207}\text{Pb}/^{206}\text{Pb}$ ratio, for which the value is close to 1. Data are normalized to the accepted values of the international standard NBS SRM 981 (National Institute of Standards and Technology), by applying a correction factor of $+0.08\%$ atomic mass unit to allow comparisons between laboratories. A measurement of the environmental lead acquired by the sample throughout the entire preparation analysis procedure was obtained in the form of a lead blank measurement. The amount of contamination detected in blanks was generally approximately 200 pg for blood and urine and <300 pg for the dietary samples. Because the blanks contributed negligibly to the lead in the sample, no blank corrections to the data were performed.

Questionnaire. An initial dietary questionnaire was administered and repeated at least once at a later date, usually coincident with conception and postpregnancy. Particular attention was directed towards diet but the questionnaire also covered such aspects as ethnic medication, cosmetics, etc. The questionnaire was supplemented on occasion by inspection of storage areas such as kitchen cupboards and refrigerators to identify any food items that may have been overlooked by the subjects.

Data analysis. Relationships between data sets were initially examined graphically for individual cases.

The data were subdivided into blood and urine for migrant female adults and Australian female adults. They were then further divided into time intervals of pre-pregnancy, pregnancy, and postpartum and the pregnancy and postpartum periods were subdivided further, where possible, into time intervals (e.g., first, second, and

third trimesters of pregnancy). It was not possible to perform analyses on all data sets, such as prepregnancy blood and urine versus diet for Australian controls, because of the limited amount of data. Blood and dietary data for migrant adults who did not conceive (nonpregnant migrant controls) were reanalyzed in the present study; these data, but not the statistical analyses applied here, have been published (18).

Best subset regression analyses were conducted to determine the best subset of diet variables (if any) to predict a blood or urine variable and then further refined using multiple linear regression analyses [Sigmastat version 2.0 (SPSS, Inc., Chicago) and SPSS version 8.0 (SPSS, Inc.)]. The variables measured were $^{208}\text{Pb}/^{206}\text{Pb}$, $^{207}\text{Pb}/^{206}\text{Pb}$, $^{206}\text{Pb}/^{204}\text{Pb}$, and Pb concentration.

The data were also analyzed using the general linear modeling procedure of SAS version 5.0 (SAS Institute Inc., Cary, NC), which permitted the estimation of means from the estimates of model parameters.

Results

Dietary samples. The mean daily dietary intake for the 29 subjects in this study was 8.5 μg Pb/day with a range of 2.7–39 μg /day. Data for 6-day duplicate dietary samples for all adult females in this study ($n = 263$; n = the number of individual samples analyzed for lead isotope ratios and lead concentrations) showed that lead concentration in diet was generally low with a mean and standard deviation (SD) of 5.8 ± 3 $\mu\text{g}/\text{kg}$ [geometric mean (GM) 5.2 $\mu\text{g}/\text{kg}$]. The isotopic composition in the diet varied considerably with $^{206}\text{Pb}/^{204}\text{Pb}$ ratios, ranging from 16.6 to 19.0 with a mean and SD of 17.56 ± 0.34 (GM 17.54). In general, the dietary intake had Australian values, although when we began this longitudinal study in 1990, the isotopic composition for

Table 1. Summary of dietary information

Parameter	Subjects	Mean	Median	SD	Min	Max	<i>n</i>
Diet Pb ($\mu\text{g}/\text{kg}$)	NPMC	5.5	5.2	2.4	0.13	15.3	92
	PM	5.8 ^a	5.0	3.0	1.3	18.7	117
	AustPC	6.8	6.5	3.2	2.8	17.3	40
Diet $^{206}\text{Pb}/^{204}\text{Pb}$	NPMC	17.47	17.41	0.31	16.57	18.86	92
	PM	17.59 ^a	17.57	0.35	16.97	18.92	117
	AustPC	17.68	17.62	0.36	17.03	19.00	40
Daily consumption (g)	NPMC	1,232	1,232	335	467	1,929	92
	PM	1,531 ^b	1,547	305	716	2,096	95
	AustPC	1,415	1,401	320	885	2,306	36
Daily intake ($\mu\text{g}/\text{kg}/\text{day}$) ^c	NPMC	6.8	5.7	3.7	0.2	25.5	92
	PM	9.0 ^a	7.4	5.6	2.7	39.2	95
	AustPC	9.1	9.0	4.2	2.7	22.4	36

Abbreviations: SD, standard deviation; NPMC, nonpregnant migrant controls; PM, pregnant migrants; AustPC, Australian pregnant controls.

^aDifference between pregnant migrant and Australian control data are not significant at $p = 0.05$.

^bDifference between pregnant migrant and Australian control data are significant at $p = 0.05$.

^cAverage intake over 6-day sampling period.

a quarterly market basket survey over a 12-month period was <17.0 (17). These changes were also observed in the slightly lower $^{206}\text{Pb}/^{204}\text{Pb}$ ratio in the diet of the nonpregnant migrant controls as compared to the pregnant migrants and Australian controls (Table 1). However, there were no statistically significant differences (t -test, two-tail) between the pregnant migrants and Australian controls for the concentration of lead in diet, the isotopic composition, or daily intake. Most of the data for the nonpregnant migrant controls were obtained earlier in this study in investigations of long-term monitoring to determine the validity of using migrant subjects (10) and to determine changes in the blood lead levels of children as compared to migrant mothers (18). Because of international trade, the food market is now global; therefore, conclusions from isotopic studies undertaken without due regard to the source and analysis of the diet (20) should be treated with caution.

The median daily intake of $7.4 \mu\text{g Pb/kg/day}$ for the female adults in this study is relatively low and compares with the estimate for a U.S. female adult of $8 \mu\text{g Pb/kg/day}$ (3). The U.S. value is, however, derived from market basket surveys with estimates of daily consumption of a broad range of items, whereas a 6-day duplicate more truly represents the individual intakes, especially for migrants whose diet preferences may vary from those of the average Australian.

Biological samples. The mean blood lead concentration for both groups was approximately $3 \mu\text{g/dl}$. The GM blood lead concentration in the migrant subjects at the time of first sampling after arrival in Australia was also $3.0 \mu\text{g/dl}$, with a range from 1.5 to 20. For the Australian controls, the range in blood lead was 1.9–4.3 $\mu\text{g/dl}$, with a GM of 3.1 $\mu\text{g/dl}$. The isotopic variations for the Australian controls ranged from 16.71 to 17.25, with a mean of 17.03 ($n = 41$). Isotopic variations for the migrant subjects depend on several factors, including time after arrival in Australia and state of pregnancy, as shown in Figures 1–3.

Lead concentrations in urine have a mean value of $2.7 \pm 3.0 \mu\text{g/kg}$ (GM = 1.8 $\mu\text{g/kg}$) and range from 0.1 to 28.1 $\mu\text{g/kg}$ ($n = 327$). As with blood samples, the isotopic variations can be large depending on the status of the mother. There is a high degree of correlation of >0.9 in the isotopic ratios between blood and urine but a much lower correlation for the lead concentrations between blood and urine (21,22).

Environmental samples (water, dust, gasoline, air). Environmental lead concentrations were consistent with measurements

from urban environments (23). Mean lead (\pm SD) levels in water were $0.91 \pm 0.84 \mu\text{g/l}$ ($n = 192$) with a mean $^{206}\text{Pb}/^{204}\text{Pb}$ of 16.55 ± 0.21 ; in house dust the levels were $86 \pm 56 \mu\text{g/m}^2/30$ days with mean $^{206}\text{Pb}/^{204}\text{Pb}$ of 16.80 ± 0.19 ($n = 164$). The variation in the lead loading in house dust is a reflection of the locality of the dwelling, especially with respect to the proximity of major traffic thoroughfares. Isotopic analyses

of gasoline and particulates collected on high-volume air filters from Sydney over the period 1986–1996 show the $^{206}\text{Pb}/^{204}\text{Pb}$ ratio ranged from 16.3 to 7.0 (24). The lead concentration in suburban Sydney air has fallen dramatically since the introduction of unleaded gasoline in 1986 and is now consistent with the levels found in U.S. cities ($0.1 \mu\text{g Pb/m}^3$) (25). The value of $0.1 \mu\text{g Pb/m}^3$ is the default number used

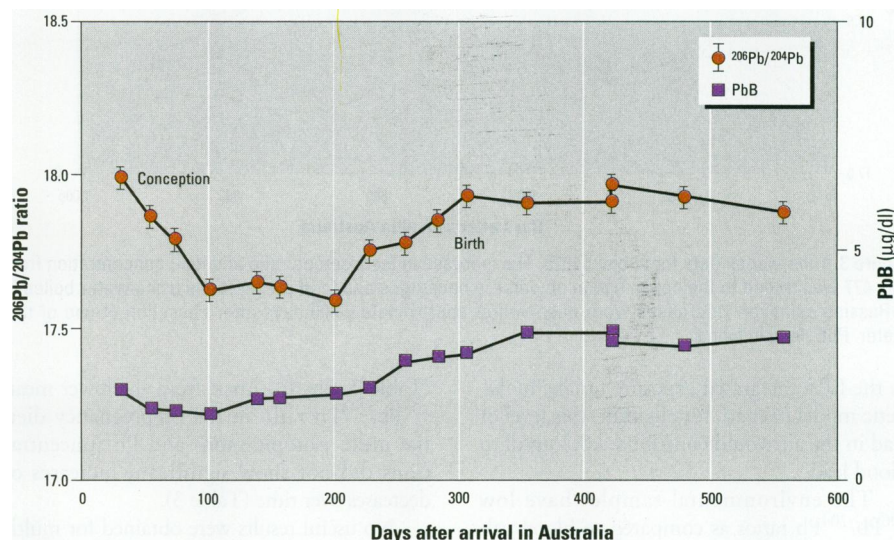


Figure 1. Time-series lead isotopic composition (as the $^{206}\text{Pb}/^{204}\text{Pb}$ ratio) and lead concentration data in blood for subject 1043, who conceived upon arrival in Australia. Initially there is a decrease in $^{206}\text{Pb}/^{204}\text{Pb}$ ratio in blood toward Australian values (approximately 17.0), then during gestation there is an increase attributed to mobilization of lead from the maternal skeleton. PbB, lead in blood. Error bars in this and the succeeding figures are 2σ errors of $\pm 0.2\%$ on the $^{206}\text{Pb}/^{204}\text{Pb}$ ratio and $\pm 3\%$ on the lead concentrations. The error bars for lead concentrations are smaller than the symbol size.

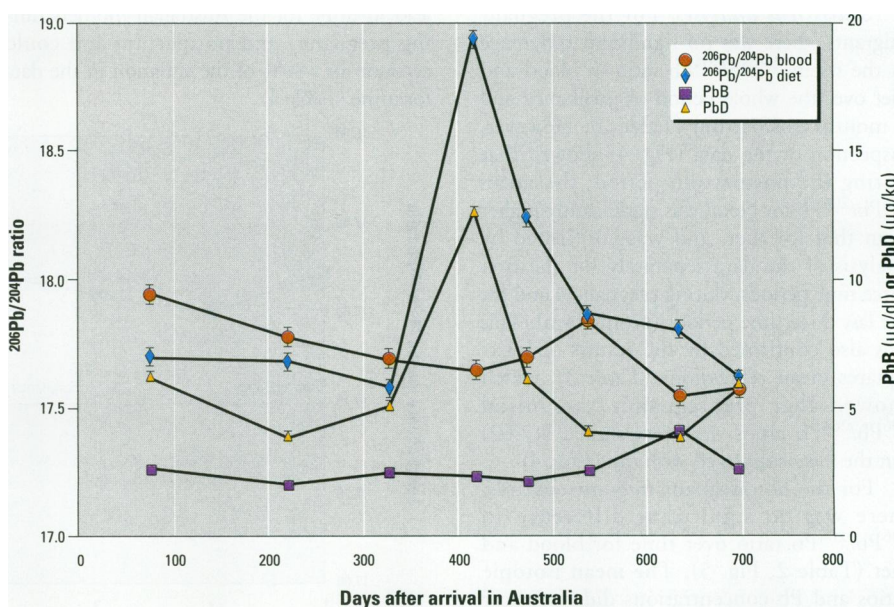


Figure 2. Time-series lead isotopic composition ($^{206}\text{Pb}/^{204}\text{Pb}$ ratio) and lead concentration data in blood and diet for subject 1055. The major spikes in isotopic composition and lead concentration for diet exhibit minimal impact on blood measures. The increases in blood measures from day 474 onward are attributed to mobilization of skeletal lead in response to lactation. Abbreviations: PbB, lead in blood; PbD, lead in diet.

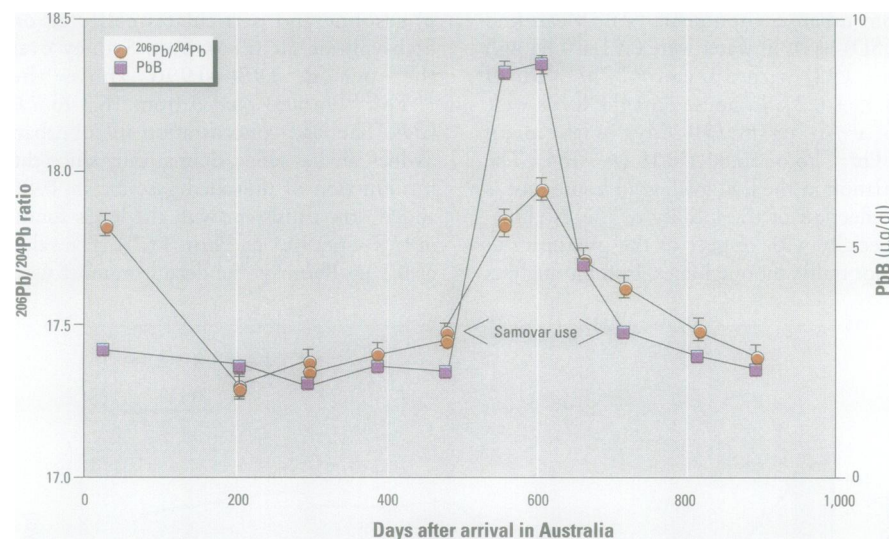


Figure 3. Time-series data for subject 1025. The increase in lead isotope ratio and lead concentration from day 477 was traced to the consumption of water in beverages and food preparations using water boiled in a Russian samovar. Decreases were observed at approximately 600 days after cessation of use of this water. PbB, lead in blood.

in the EPA integrated exposure uptake biokinetic model (1) and, for an adult, this level of lead in the air would contribute $<0.2 \mu\text{g/dl}$ to blood lead.

The environmental samples have low $^{206}\text{Pb}/^{204}\text{Pb}$ ratios as compared to blood values of the migrant subjects but are similar to those observed in blood of the Australian controls. The impact of the environmental samples would be to decrease the $^{206}\text{Pb}/^{204}\text{Pb}$ ratio in blood of migrant subjects with $^{206}\text{Pb}/^{204}\text{Pb}$ higher than the environmental samples.

Statistical analyses. For the pregnant migrants, there was no significant difference in the mean $^{206}\text{Pb}/^{204}\text{Pb}$ ratio in blood and diet over the whole period of pregnancy and 6 months postpartum (Table 2). However, inspection of the data (Fig. 4) showed that during the postpartum period, the mean $^{206}\text{Pb}/^{204}\text{Pb}$ for blood was significantly higher than that for diet, and was confirmed by analysis of the data separately for the first three time periods (during pregnancy) and for the last three time periods (postpartum). This was also confirmed by the results of least-squares mean regressions (Table 3), which showed that pre-pregnancy the initial $^{206}\text{Pb}/^{204}\text{Pb}$ ratios were very similar (17.62) but the means diverged with time (Fig. 4).

For the nonpregnant migrant controls, there was no significant difference in $^{206}\text{Pb}/^{204}\text{Pb}$ ratio over time for blood and diet (Table 2, Fig. 5). The mean isotopic ratios and Pb concentrations did not show significant increases or decreases over time (Table 3).

For the pregnant Australian subjects, there was a statistically significant difference in $^{206}\text{Pb}/^{204}\text{Pb}$ ratio over time for blood and diet

(Table 2, Fig. 6). Apart from the lower mean $^{206}\text{Pb}/^{204}\text{Pb}$ ratio in the pre-pregnancy diet, the mean isotopic ratios and Pb concentrations did not show significant increases or decreases over time (Table 3).

No useful results were obtained for multiple linear regression analyses with dietary isotopic composition and lead concentration as the independent variables and blood as the dependent outcome over various time periods for the migrant groups. Dietary isotopic composition and lead concentration were significant predictors of isotopic composition and lead in urine for the Australian mothers during pregnancy and postpartum and could account for $>80\%$ of the variation in the data for urine (Table 4).

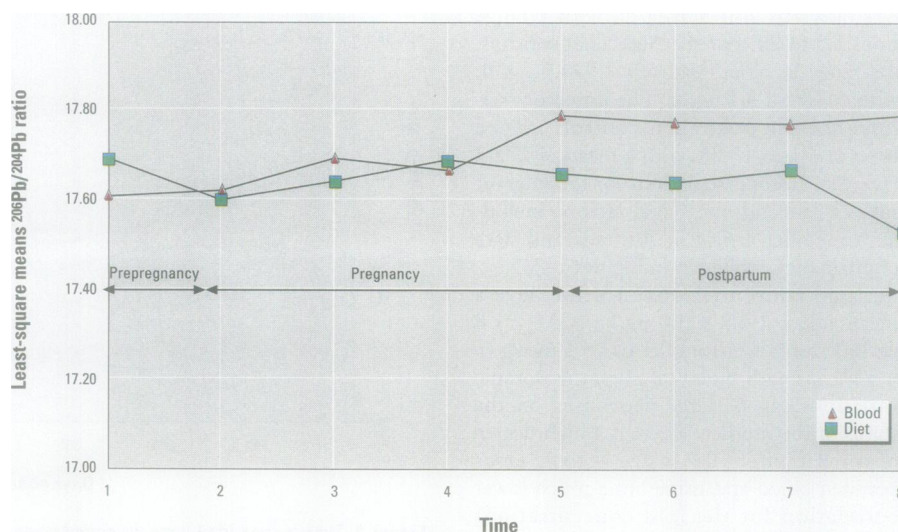


Figure 4. Estimated marginal means for the $^{206}\text{Pb}/^{204}\text{Pb}$ ratio in blood and diet for migrant subjects during pregnancy and postpartum, showing the increase in $^{206}\text{Pb}/^{204}\text{Pb}$ ratio in blood, especially during postpartum; the increase is attributed to increased mobilization of lead from the maternal skeleton.

Table 2. Results for analyses of variance for blood and diet

Subjects	n	p		
		$^{207}\text{Pb}/^{206}\text{Pb}$	$^{206}\text{Pb}/^{204}\text{Pb}$	Pb
PM	14	0.56	0.60	<0.005
NPM	8	0.42	0.42	<0.005
AustPC	5	0.004	0.003	0.02

Abbreviations: PM, pregnant migrants; NPM, nonpregnant migrant controls; AustPC, Australian pregnant controls.

Graphic presentation for individuals. The impact of diet on blood can also be evaluated using time-series presentations for individuals (Fig. 2) in contrast to conventional statistical treatments, where the combined data may camouflage relationships between diet and blood. In most cases, even where there is a major spike in lead isotopic composition and lead concentration in the 6-day duplicate diet, there are negligible changes in blood lead isotopic composition and lead concentration. Several examples similar to that in Figure 2 have been published (18). On the other hand, there were rare individual cases in which a direct relationship was observed between diet and blood lead concentrations, as shown in Figure 3 for subject 1025, a nonpregnant migrant control. The spike was traced to a Russian samovar containing lead solder; the hot water from the samovar was used in beverage and other food preparations. Cessation of use of the samovar resulted in a return to normal isotopic composition and blood lead. The significant changes of lead concentration and isotope ratios observed in the blood of this subject may point to an increased bioavailability of lead in warm to hot water as compared to other foodstuffs. Elevated blood lead in an individual was also traced to a samovar (26).

Discussion

Relevance to skeletal mobilization. Our results are based on three groups of female adults currently living in Australia and whose bone stores of lead acquired between the ages of 0–35 years are from isotopically different sources. Upon their arrival in Australia, the migrant subjects' blood lead isotope values are taken to be similar to those of the bone-stored values because of the equilibrium existing between bone and blood lead over the typical lifetime of humans (27). An indirect measure of the isotopic composition of the mother's bone can be obtained from analysis of her infant's teeth, if available (28). Blood lead values then decrease toward equilibrium with Australian lead values (Fig. 1). Our estimates of rates of exchange between skeletal lead and environmental lead for these subjects (10,29) are consistent with clearance rates of lead from blood obtained for male subjects (30,31).

Equilibrium between the isotopic composition in migrant subjects' blood and Australian environmental lead was reached after approximately 4–6 months in Australia (10). During pregnancy, as bone lead stores are mobilized, blood lead isotope values increase again (Fig. 1), a change that we believe reflects the presence of lead that has been released from bone stores (11,12). The migrant group acquired bone stores of lead with $^{206}\text{Pb}/^{204}\text{Pb}$ isotopic ratios ranging from 17.7 to 18.5, which are unlike those acquired by the Australian-born subjects (approximately 17.0), allowing a distinction to be made between current lead in blood acquired from Australian sources and older bone-stored lead values not available from Australian sources.

Using this approach of high precision lead isotope fingerprinting, we have argued previously that the dominant contribution to blood lead in recently arrived migrants to Australia was from skeletal sources and that this contribution ranged from 41 to 73%, even after more than 300 days (10). Furthermore, we proposed that during pregnancy and the postpregnancy period, extra lead was mobilized from the maternal skeleton, with larger contributions during the postpregnancy period as a consequence of higher bone turnover associated with the low calcium intake (12). We do not consider that seasonal effects play a significant role in the observed changes for lead isotopic composition and lead concentration because of the monitoring of the nonpregnant control group for periods of up to 2 years (18).

It was not possible to detect skeletal contributions in an Australian pregnant control group because the lead in their skeletons had the same isotopic composition as the Australian environment (including diet) prevailing at that time. In the early 1990s the $^{206}\text{Pb}/^{204}\text{Pb}$ ratio in the Australian diet was

Table 3. Linear regressions of $^{206}\text{Pb}/^{204}\text{Pb}$ ratio and lead concentration on time

Subjects	$^{206}\text{Pb}/^{204}\text{Pb}$			Pb		
	Ratio and SE	t^a	p^b	Conc and SE	t	p
Pregnant migrants						
Blood	17.580 ± 0.032	3.99	<0.001	2.25 ± 0.09	2.14	0.04
Diet	17.620 ± 0.008	0.62	0.54	6.43 ± 0.01	-0.06	0.95
Nonpregnant migrants						
Blood	17.423 ± 0.037	1.66	0.11	2.53 ± 0.10	0.49	0.63
Diet	17.359 ± 0.011	0.37	0.72	5.80 ± 0.00	-0.01	1.0
Australian pregnant controls						
Blood	17.044 ± 0.004	0.43	0.67	3.38 ± 0.04	0.39	0.70
Diet	17.630 ± 0.041	1.41	0.17	6.20 ± 0.05	0.29	0.78

Abbreviations: Conc, concentration; SE, standard error.

^a t is the t -statistic.

^b p is the associated p -value to test whether the true regression slope is 0.

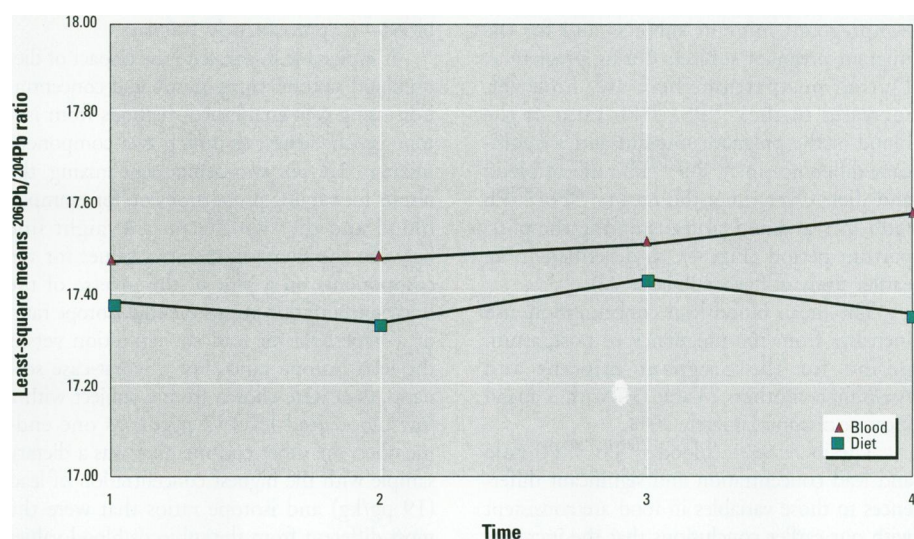


Figure 5. Estimated marginal means for the $^{206}\text{Pb}/^{204}\text{Pb}$ ratio in blood and diet over a 2-year period for migrant subjects who did not conceive.

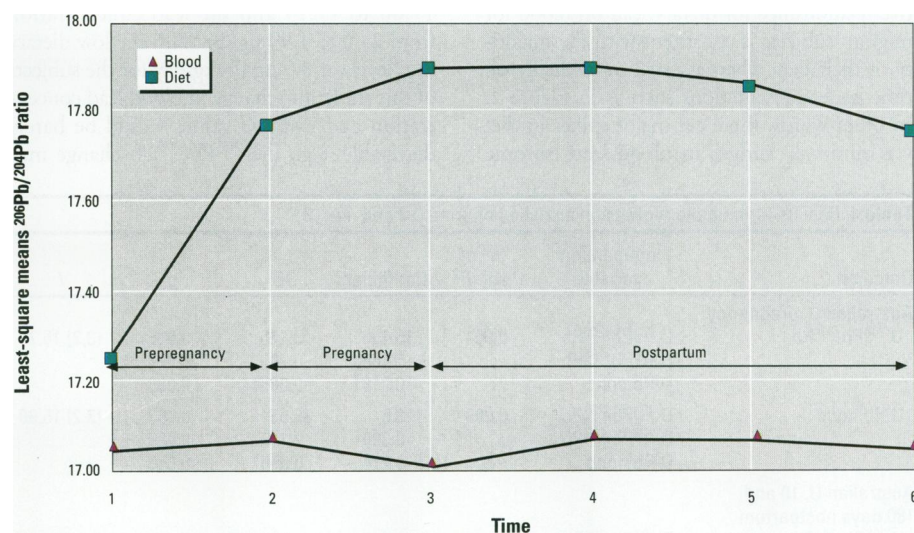


Figure 6. Estimated marginal means for the $^{206}\text{Pb}/^{204}\text{Pb}$ ratio in blood and diet for Australian subjects during pregnancy and postpartum.

<17.0 (17), but at the time of this study it was approximately 17.6 (Tables 3 and 4, Fig. 6). If dietary contribution to blood and urine was significant, it should be readily detectable using our methods. The statistical analyses show that over time there is no significant change in mean values for the Australian subjects (Table 3), but that there is a significant difference in the isotopic composition of blood and diet (Fig. 6). It was argued earlier (18) that the negligible changes in isotopic composition of blood for migrant subjects in response to major changes in dietary isotopic composition (e.g., Fig. 2) indicate a small lead contribution from diet to blood.

In contrast to Australian subjects, there are no significant differences in isotopic composition for diet and blood over time for nonpregnant migrant subjects and for the migrant pregnant subjects during pregnancy. During postpartum there are, however, increases in the $^{206}\text{Pb}/^{204}\text{Pb}$ ratio of the blood of the pregnant migrant and a significant difference in $^{206}\text{Pb}/^{204}\text{Pb}$ ratio of blood and diet. Over time, the mean $^{206}\text{Pb}/^{204}\text{Pb}$ ratio increases and plateaus during the postpartum period (Fig. 4), as described in an earlier study of fewer subjects (12).

The mean blood lead concentration also increases from the pregnancy to postpartum period for the pregnant migrant and Australian mothers (Table 3), with a mean increase of approximately 20%.

The increases in blood $^{206}\text{Pb}/^{204}\text{Pb}$ ratio and lead concentration and significant differences to those variables in food are consistent with our earlier conclusions that the increases reflect increased mobilization from the maternal skeleton during pregnancy and lactation especially associated with a low calcium intake for the subjects (11,12).

Impacts on blood lead. The lack of predictive relationships for dietary lead on blood for migrant subjects is consistent with the majority of their data when assessed individually on time-series presentations such as in Figure 2. In other words, there are major spikes in diet yet minimal changes in blood lead isotopic

composition and concentration. There are, of course, exceptions, as in the case of subject 1025 and the use of the samovar (Fig. 3).

The potential changes in blood lead concentration from changes in dietary intake may be relatively small in real terms. For example, if the dietary intake is increased from 7 to 14 $\mu\text{g Pb/day}$ and other inputs remain constant, the blood lead increase is only 0.1–0.2 $\mu\text{g/dl}$ and would not be detectable by most analytical methods except isotope dilution. Minor spikes in either dietary isotopic composition or lead concentration may also be undetectable because of the low amount of absorption and the clearance rate of lead from blood. The mean life of lead in blood is approximately 30 days (30,31) and the circulating life for red blood cells—the main store for lead in blood—is approximately 100 days.

It is possible to calculate the impact of diet on blood isotopic composition and concentration using well-established methods from isotope geochemistry, assuming two-component mixing (32). For two-component mixing, the results for varying proportions of, for example, blood and diet will lie on a straight line between the two end-member values for the components on a plot of the inverse of the lead concentration versus the lead isotope ratio or a hyperbola for lead concentration versus the lead isotope ratio. For a worst-case scenario, data were chosen from a subject with a low blood lead level (2 $\mu\text{g/dl}$) as one end-member; the other end-member was a dietary sample with the highest concentration of lead (19 $\mu\text{g/kg}$) and isotope ratios that were the most different from the subject's blood values ($^{206}\text{Pb}/^{204}\text{Pb}$ of 17.0 for blood and $^{206}\text{Pb}/^{204}\text{Pb}$ of 19.0 for the diet samples) (Fig. 7). Using 10% fractional absorption, the impact of this diet on the subject's blood would change the $^{206}\text{Pb}/^{204}\text{Pb}$ ratio from 17.00 to 17.19 and the lead concentration from 2.00 to 1.99 $\mu\text{g/dl}$. With the low dietary lead levels of 8.5 $\mu\text{g Pb/kg/day}$ for the subjects of this study, the change in blood lead concentration and isotopic values would be barely detectable; e.g., the $^{206}\text{Pb}/^{204}\text{Pb}$ change may

be from 17.00 to 17.02. The value of 17.0 is the mean value for blood of the Australian subjects (Table 3). The minimal changes shown by the mixing calculations are consistent with the statistical analyses for the Australian subjects, where the blood shows little change over time in spite of the high $^{206}\text{Pb}/^{204}\text{Pb}$ ratio in the diet (Fig. 6). Hence, we infer that the fractional absorption rate from the adult's diet is <10% and any dietary contribution to blood lead is overwhelmed by skeletal lead, especially during pregnancy and postpartum.

Clearance rates of lead from blood may also contribute to the lack of correlation between lead and blood in the migrant subjects. Inskip et al. (33) traced the clearance rate of lead from soft tissue by administering enriched isotopes (in our human study this would be equivalent to Australian sources of lead such as diet) to monkeys with a previous long-term exposure to lead from a source different from that of the administered lead (equivalent to migrant lead in the human study). Using unmixing relationships, the authors were able to distinguish between the oral dose and the historic/recent lead that was derived from bone. The flux of lead released from bone to blood was relatively constant and was dominated by historic bone stores. The study showed that the majority of the administered enriched isotopes were rapidly cleared from soft tissues, with very small amounts of enriched isotopes observed in blood samples taken 23 months after the last dose of enriched isotopes. Hence, in our subjects, although the majority of the dietary spike would be mostly cleared from the blood

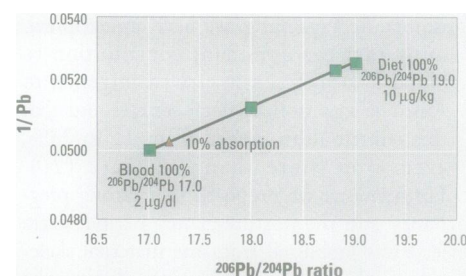


Figure 7. Graphic presentation of lead concentration⁻¹ versus the $^{206}\text{Pb}/^{204}\text{Pb}$ ratio illustrating the two-component mixing method for estimating impact of dietary intake on blood lead isotopic composition and lead concentration for a worst-case scenario of low blood lead and high dietary lead concentrations. The high-lead diet also has a $^{206}\text{Pb}/^{204}\text{Pb}$ ratio of 19.0, markedly different from the blood $^{206}\text{Pb}/^{204}\text{Pb}$ ratio of 17.0. Each end point is taken as a pure end-member consisting of 100% of the parameter so that the end point for blood has a $^{206}\text{Pb}/^{204}\text{Pb}$ ratio of 17.0 and a blood lead concentration of 2 $\mu\text{g/dl}$ and the pure end-member for diet (100%) has a $^{206}\text{Pb}/^{204}\text{Pb}$ ratio of 19.0 and a blood lead concentration of 19 $\mu\text{g/kg}$. (The difference in $^{206}\text{Pb}/^{204}\text{Pb}$ ratio of 19 and 17 is >60 times our experimental error).

Table 4. Results for multiple regression analyses of urine (U) and diet (D)

Outcome	Independent variable	R^2 or adj. R^2	Coefficient	SE	p	f
Australian U, pregnancy	D $^{208}\text{Pb}/^{206}\text{Pb}$	0.904	16.193	3.165	0.036	(3,2) 16.7
	D $^{206}\text{Pb}/^{204}\text{Pb}$		1.168	0.203	0.029	
	D Pb conc		0.0181	0.00544	0.080	
U Pb conc	D $^{208}\text{Pb}/^{206}\text{Pb}$	0.900	129.6	32.964	0.059	(3,2) 15.99
	D $^{206}\text{Pb}/^{204}\text{Pb}$		8.389	2.117	0.058	
	D Pb conc		0.274	0.0567	0.040	
Australian U, 10 and 180 days postpartum	D $^{207}\text{Pb}/^{206}\text{Pb}$	0.809	27.50	7.7006	0.038	(1,3) 12.74
	D Pb conc		0.290	0.0477	0.026	

Abbreviations: adj, adjusted; conc, concentration.

and soft tissues, the small amount taken up by trabecular bone may still be detectable several months later. Because changes were detected in blood isotopic composition and blood lead after a spike of much less intensity than for the worst-case scenario illustrated in Figure 7, both in the quarter following the spike and thereafter, these observations appear consistent with the small contribution of lead from diet to blood in adults. Information on absorption rates provided by other studies (31,34) is also relevant to our results. Chamberlain (31) summarized the data for absorption of soluble tracer lead taken with meals and obtained a weighted mean value of 8%. Our data are consistent with these estimates. Furthermore, the long-term absorption studies of Rabinowitz et al. (34) were carried out for 41–116 days so that the time intervals are relevant to our investigations, which lasted in some cases for more than 2 years.

There are some limitations to assessing dietary lead at fixed points in time and then comparing the blood lead and isotopic composition measurements at the same point in time. One of the limitations is that blood would reflect the contributions (if any) at some time after, and not concurrent with, the assessment of diet. Blood was sampled at the beginning of the 6-day dietary sampling period, when dietary sample containers were delivered. To test the effect of timing of diet and blood sampling, we undertook a small study at the inception of this project by collecting blood samples prior to and at the end of the dietary sampling period. We found negligible differences in lead isotopic composition or lead concentration values. Furthermore, questioning of the subjects indicated that they had an approximate 14-day shopping menu with only seasonal fruit variations over the whole year. Another problem may be that newly changed isotopic inputs from diet may not impact red blood cell isotopic proportions as immediately, or to the degree expected, because possibly up to half of the lead in red cells is less exchangeable and may remain with the cell for its circulating life of about 100 days. Thus newly formed cells or plasma lead should reflect concurrent isotopic dietary and skeletal contributions, but they make up only a few percent of the red cell mass. A reflection of current dietary contributions to plasma was proposed as the reason for the more rapid changes toward Australian values observed in urine as compared to blood samples for many of the migrant subjects in their first 4–6 months after arrival in Australia (10,21). The impact of short-term dietary contributions to blood lead are also illustrated by the data for subject 1025, who consumed water from a samovar (Fig. 3). The rapid increases in lead concentration over the period from 477 to

554 days and then similar rapid decreases after cessation of the consumption of this water at about 600 days and a return to a baseline may be an indication that the lead has been incorporated into newly formed cells.

In conclusion, by using a reliable measure of dietary intake and high precision analytical techniques, we suggest that consumption of a diet containing average amounts of lead contributes minimally to blood lead in adult females. We suggest that the dietary and blood and urine relationships observed in the migrant subjects during pregnancy and lactation are consistent with earlier conclusions that the changes in lead isotopic composition and increases in blood lead concentration arise from mobilization of lead from bone stores.

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